

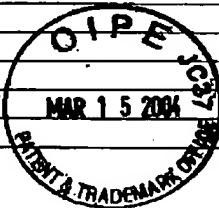
Appendix A

## TO THE U.S. PATENT AND TRADEMARK OFFICE

## TO THE U.S. PATENT AND TRADEMARK OFFICE

Please stamp the date of receipt of the following document(s) and return this card to us:

INVENTOR(S):	Fitzgerald et al.
RE:	PATENT APPLN. FILED 2/17/00 FOR "RECOMBINANT ANTIBODIES AND IMMUNOCONJUGATES TARGETED TO CD-22 BEARING CELLS AND TUMORS"
TITLE OF DOCUMENT(S):	Supplemental Response; Declaration of Dr. David J. Fitzgerald Under 37 CFR 1.132"; Transmittal Form PTO/SB/21.
Application No.	09/381,497
File No.	15280-317-100
Date Due	
Date Mailed	11 March 2004
Attorney/Secretary	JML/mcd



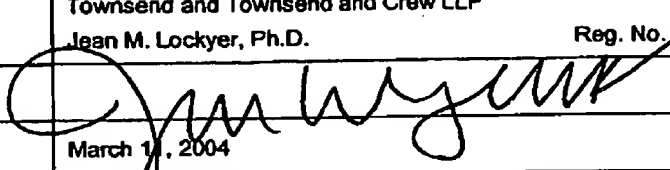
return this card to us:

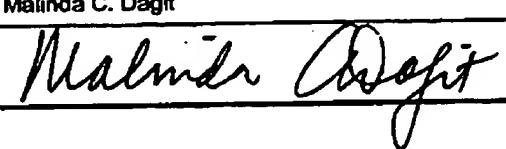
REBINANT  
TARGETED TO CD-22

der 37 CFR 1.132";

<b>TRANSMITTAL FORM</b> <i>(to be used for all correspondence after initial filing)</i>		Application Number	09/381,497
		Filing Date	February 17, 2000
		First Named Inventor	FITZGERALD, David J.
		Art Unit	1642
		Examiner Name	Helms, Larry R.
Total Number of Pages in This Submission		Attorney Docket Number	015280-317100US

ENCLOSURES (Check all that apply)		
<input type="checkbox"/> Fee Transmittal Form <input type="checkbox"/> Fee Attached <input checked="" type="checkbox"/> Amendment/Reply- "Supplemental Response" <input type="checkbox"/> After Final <input checked="" type="checkbox"/> Affidavits/declaration(s)- "Declaration of Dr. David J Fitzgerald Under 37 CFR 1.132" <input type="checkbox"/> Extension of Time Request <input type="checkbox"/> Express Abandonment Request <input type="checkbox"/> Information Disclosure Statement <input type="checkbox"/> Certified Copy of Priority Document(s) <input type="checkbox"/> Response to Missing Parts/ Incomplete Application <input type="checkbox"/> Response to Missing Parts under 37 CFR 1.52 or 1.53	<input type="checkbox"/> Drawing(s) <input type="checkbox"/> Licensing-related Papers <input type="checkbox"/> Petition <input type="checkbox"/> Petition to Convert to a Provisional Application <input type="checkbox"/> Power of Attorney, Revocation Change of Correspondence Address <input type="checkbox"/> Terminal Disclaimer <input type="checkbox"/> Request for Refund <input type="checkbox"/> CD, Number of CD(s)	<input type="checkbox"/> After Allowance Communication to Group <input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences <input type="checkbox"/> Appeal Communication to Group (Appeal Notice, Brief, Reply Brief) <input type="checkbox"/> Proprietary Information <input type="checkbox"/> Status Letter <input checked="" type="checkbox"/> Other Enclosure(s) <i>(please identify below):</i> Return Postcard
Remarks	The Commissioner is authorized to charge any additional fees to Deposit Account 20-1430.	

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT		
Firm or Individual	Townsend and Townsend and Crew LLP Jean M. Lockyer, Ph.D. Reg. No. 44,879	
Signature		
Date	March 11, 2004	

CERTIFICATE OF TRANSMISSION/MAILING			
I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below.			
Typed or printed name	Malinda C. Dagitt		
Signature		Date	11 March 2004

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Commissioner for Patents  
P.O. Box 1450  
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On

11 March 2004

TOWNSEND and TOWNSEND and CREW LLP

By:

Malinda AdgeyPATENT

Attorney Docket No.: 015280-317100US  
Client Ref. No.: DHHS Ref. No.: E-059-97/1

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of:

FitzGerald et al.

Application No.: 09/381,497

Filed: February 17, 2000

For: RECOMBINANT ANTIBODIES  
AND IMMUNOCONJUGATES  
TARGETED TO CD-22 BEARING  
CELLS AND TUMORS

Customer No.: 20350

Confirmation No. 4036

Examiner: Larry R. Helms, Ph.D.

Technology Center/Art Unit: 1642

SUPPLEMENTAL RESPONSE

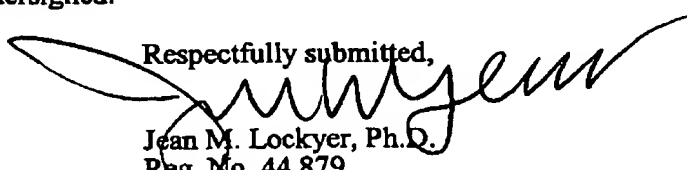
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

Supplemental to Applicants' Amendment mailed for filing on February 20, 2004,  
Applicants respectfully request entry of the Rule 1.132 Declaration of Dr. David J. Fitzgerald  
submitted herewith.

If the Examiner believes a telephone conference would expedite prosecution of  
this application, please telephone the undersigned.

Respectfully submitted,

  
Jean M. Lockyer, Ph.D.  
Reg. No. 44,879

TOWNSEND and TOWNSEND and CREW LLP  
Two Embarcadero Center, Eighth Floor  
San Francisco, California 94111-3834  
Tel: 415-576-0200

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**PATENT**  
Attorney Docket No.: 015280-317100US  
Client Reference No.: E-059-97/1f

Assistant Commissioner for Patents, ~~PO Box 1450~~  
~~Washington, D.C. 20231~~ *Arlington, VA 22213-1450*

On *11 March 2004*

TOWNSEND and TOWNSEND and CREW LLP

By: *Malinda Dejit*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

FitzGerald et al.

Application No.: 09/381,497

Filed: September 20, 1999

For: RECOMBINANT ANTIBODIES  
AND IMMUNOCONJUGATES  
TARGETED TO CD-22 BEARING  
CELLS AND TUMORS

Examiner: Larry R. Helms, Ph.D.

Art Unit: 1642

DECLARATION OF DR. DAVID J.  
FITZGERALD UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

I, Dr. David J. FitzGerald, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. I received a Ph.D. in Microbiology in 1982 from the University of Cincinnati, College of Medicine, in Cincinnati Ohio.
2. I am currently employed as the Chief of the Biotherapy Section, Laboratory of Molecular Biology in the Division of Basic Science of the National Cancer Institute at the National Institutes of Health where I conduct research relating to immunotoxins. I have authored

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over 170 peer-reviewed scientific publications and chapters in this area. A copy of my curriculum vitae was previously submitted with Applicants' response filed May 2, 2001.

3. I have read and am familiar with the contents of the application. The claims currently at issue are drawn to a recombinant immunoconjugate that comprises a disulfide-stabilized RFB4 binding fragment linked to a therapeutic moiety. I understand that the Examiner has rejected the claims based upon his belief that the claimed recombinant immunoconjugates are obvious over the prior art. In particular, the Examiner alleges that the sequences of the RFB4 heavy and light chains were obvious in view of the existence of the known RFB4-producing hybridoma and techniques to obtain the  $V_H$  and  $V_L$  nucleic acid sequences. Further, he argues it would have been obvious to use these nucleic acid sequences to produce the dsFv-containing immunoconjugates in view of art describing the construction of dsFv antibodies. In this Declaration, I will present evidence that RFB4-containing immunoconjugates have superior expression characteristics and stability in comparison to a recombinant anti-CD22 immunoconjugate containing a different anti-CD22 antibody.

X Furthermore, this in this Declaration, I attest to the surprising binding characteristics and cytotoxicity of the claimed immunotoxin.

4. RFB4 immunoconjugates are generated by recombinant technology. Thus, the RFB4 component must express well. As one of skill in the art, the RFB4  $V_H$  and  $V_L$  sequences are expressed surprisingly well and recombinant conjugates generated using them exhibit superior binding properties. In contrast, we have previously attempted to construct another recombinant anti-CD22 immunoconjugate using sequences from a different antibody, LL2. The LL2  $V_H$  and  $V_L$  regions were very difficult to express and moreover, recombinant LL2-PE38 immunoconjugate exhibited poor cytotoxicity.

5. We first attempted to construct a single chain (sc) LL2 binding fragment. The genes encoding the  $V_H$  and  $V_L$  variable domains were obtained by PCR using primers to the known sequence. Restriction sites for assembling the peptide linker sequence,  $(Gly_4Ser)_3$ , that connects the  $V_H$  and  $V_L$  domains and for cloning into the expression vector were also introduced

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by PCR. An expression vector was created that contained a CD22 V<sub>H</sub>-linkerV<sub>L</sub>-PE38 fusion construct. The expression plasmids were expressed in *E. coli* BL21 ( $\lambda$ DE3). The yield of immunotoxin obtained was very low. Moreover, cytotoxicity of the small amount of immunotoxin that was obtained was very poor.

6. Cytotoxicity was evaluated using CA46 and Daudi Burkitt's lymphoma cells. The IC<sub>50</sub> value, the concentration of immunotoxin that caused a 50% inhibition of protein synthesis, was determined after a 20-hour incubation with the toxin. The scLL2-PE38 immunoconjugate showed an IC<sub>50</sub> of 1  $\mu$ g/ml for both CA46 and Daudi cells. Attempts were made to increase both the yield of the expressed product and the cytotoxicity of the immunotoxin. These changes resulted in only a slight improvement in expression. Cytotoxicity of this conjugate was also somewhat improved, but still exhibited an IC<sub>50</sub> of only about 250 ng/ml. Our attempts to produce a recombinant ds(Fv) LL2 immunotoxin also failed due to the poor expression characteristic of the individual variable chains.

7. In contrast, RFB4 immunotoxin is expressed at much higher levels, is stable, and has superior binding characteristics and superior toxicity. These properties are unpredictable. First, it is acknowledged in the art that unknown parameters influence the degree of expression of the variable chain regions of different antibodies. Such parameters include the epitope that an antibody binds, and the folding properties of the recombinant antibodies. The art cannot predict which antibody sequence will express well or be stable, and hence, which immunotoxins can be produced at high levels.

8. Second, RFB4 immunotoxin, *e.g.*, RFB4ds(FV)-PE38, not only expresses well, but also retains the binding specificity and affinity of RFB4 IgG. This is unusual and surprising, not only in contrast to LL2-containing immunoconjugates, but in comparison to many recombinant immunotoxins. Typically, binding affinity is lowered in comparison to the parent antibody.

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9. Last, the toxicity of the recombinant RFB4ds(FV)-PE38 was over 100 times better than any immunotoxin that could be produced using LL2 as the binding moiety. Further, this immunotoxin showed potent antitumor activity not only in animal models, but also in human Phase I trials, as described in my previous Declaration, already of record, signed May 15, 2001.

10. In summary, the high level of expression, retention of parental IgG binding affinity, and superior toxicity and efficacy of RFB4ds(FV)-PE38 is surprising and cannot be predicted from the art.

11. All statements herein made of my own knowledge are true and statements made on information or belief are believed to be true. The experimental work described herein was either conducted by myself or by a co-inventor, Dr. Ira Pastan or Dr. Robert Krietman, or under our direction.

Dated: March 11<sup>th</sup>, 2004

David J. Fitzgerald  
David J. FitzGerald, Ph.D.